Precipitation of the Collagen Components by Salts

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The precipitation of native soluble collagen by salts has been applied in several purification procedures.[1] Denatured collagen also can be divided into two fractions by salt precipitation.[2] We now report further studies on the effects of salt concentration, pH and temperature.

Acid-soluble collagen was prepared from rat-tail tendon fibres by extraction with 0.5 N acetic acid at +4°C overnight. The collagen solution was cleared by centrifugation for 60 min at 35 000 *g*, dialyzed against water and lyophilized.

Before precipitation, the solutions were heated to various temperatures for 15 min. Prewarmed NaCl-solution was added to final concentrations of 5, 10, 15 (or 2.5 M), and 20 % (w/v). After 15—30 min interval the samples were cooled to room temperature and centrifuged as above. Both the suspended precipitates and the supernatants were dialyzed against water, lyophilized and dissolved in electrophoretic buffer. The electrophoretic procedure has been described separately.[3] Hydroxyproline was determined according to Neuman and Logan.[4]

The proportion of precipitated collagen and the gel-electrophoretic patterns of the supernatant and precipitate in 2.5 M NaCl varied according to pH (Figs. 1 and 2). At pH 4.6—4.8, which is most suitable for the fractionation of collagen components with ion-exchange techniques or with gel-electrophoresis, the precipitation is minimal. At pH 4.4 several components remain in the supernatant, at pH 3.6 only some α1 component remains. The separation of α1 and α3 in precipitated collagen is especially evident at pH 4.4. In the range of temperatures +4° to +30°C 96—88 % of the total collagen was precipitated, but above the denaturation point in the range +35°C to +65°C, 60—70 % only.

The final concentrations of salt influenced the precipitation, for example at +45°C, as follows: at NaCl concentrations of 5, 10, 15, and 20 % (w/v), the percentages of precipitated collagen were 2, 5, 70,

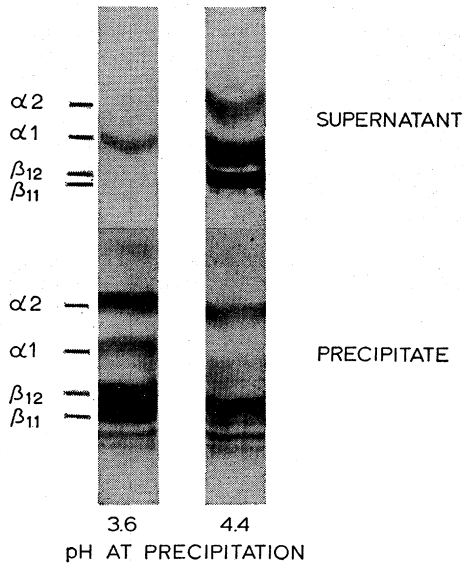

Fig. 1. Starch-gel electrophoretic patterns of collagen in the precipitate and supernatant obtained by addition of NaCl (final concn. 2.5 M) to a solution of acid-soluble collagen (final concn. 0.1 %) at pH 3.6 and 4.4. The mixture was kept at +40°C for 30 min before centrifugation.

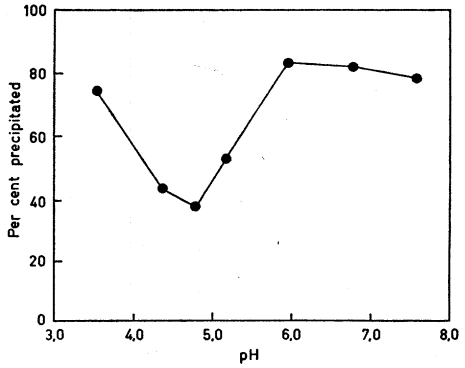

Fig. 2. Salt precipitation of rat-tail tendon collagen at varying pH. The conditions are described in the legend of Fig. 1. In the range of pH 6.0—7.6, phosphate buffer (1/15 M) was used. In the lower range, acetate buffer was used.

and 90 %, respectively. Above +35°C the amount of precipitate is more dependent on salt concentration than on temperature. At lower salt concentrations and at higher temperatures the β- and α-components are precipitated preferentially and preparative schemes may be worked out on that basis. Collagen which had been renatured at +4°C overnight behaved like native collagen on salt precipitation.

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